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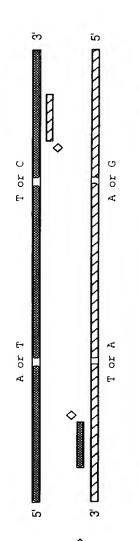
2

T or A

A or G

# PCR/ LDR

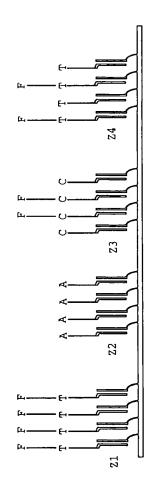
 PCR amplify region(s) containing mutations using primers, dNTPs and 7aq polymerase.



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2. Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

 Capture fluorescent products on addressable array and quantify each allele.

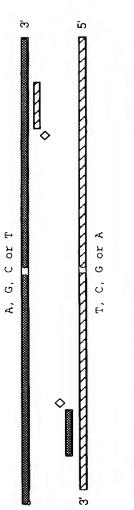


Homozygous: T allele only.

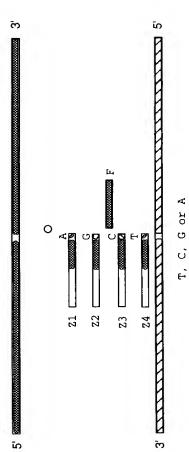
Heterozygous: C and T alleles.

# PCR/ LDR

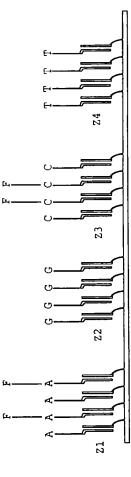
 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.



2. Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each ailele.

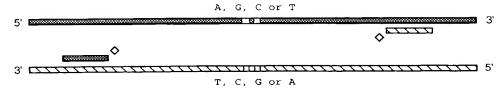


Heterozygous: A and C alleles.

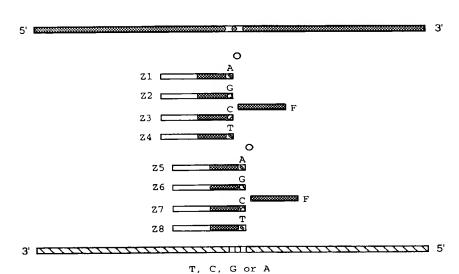
IFIIG. 4

#### PCR/LDR: N arby alleles

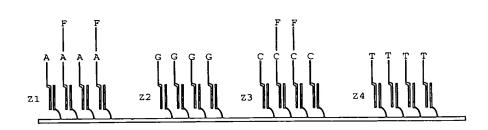
 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.



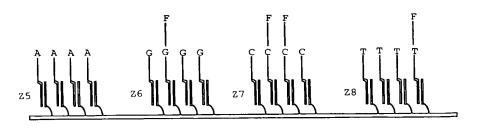
2. Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A and C alleles.



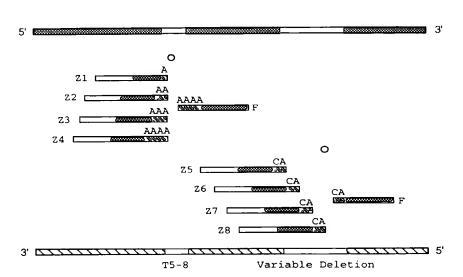
Heterozygous: G,C, and T alleles.

#### PCR/ LDR: Insertions and Deletions

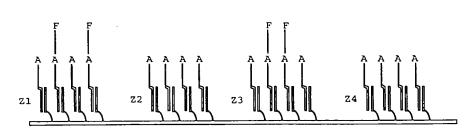
- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.
- A5-8 Variable Deletion in (CA)n

  5'

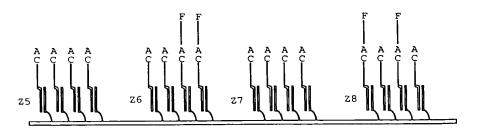
  T5-8 Variable Deletion in (GT)n
- Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



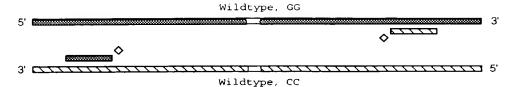
Heterozygous: A5 and A7 alleles.



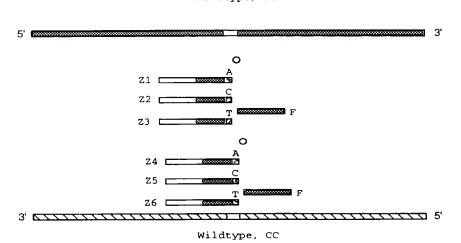
Heterozygous: (CA)5 and (CA)3 alleles.

#### PCR/ LDR: Adjacent alleles, cancer detection

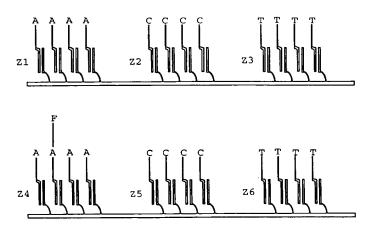
 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.



 Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



Gly to Asp mutation

#### PCR/ LDR: Nearby alleles

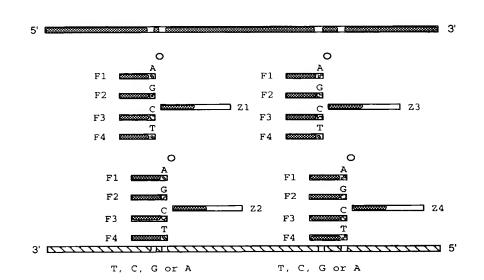
- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.
- A, G, C or T

  A, G, C or T

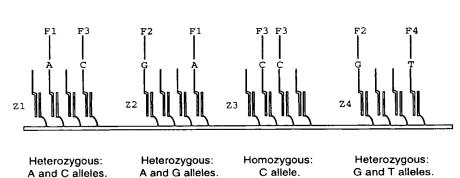
  3'

  T, C, G or A

  T, C, G or A
- Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



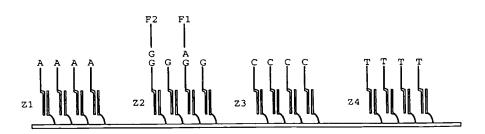
 Capture fluorescent products on addressable array and quantify each allele.



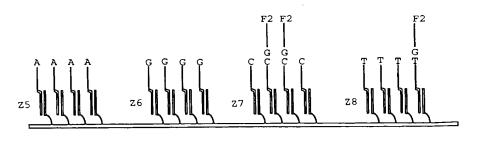
#### PCR/ LDR: Adjacent and Nearby alleles

- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase. ◊
- Wildtype, GG Wildtype, CG

  Wildtype, CC Wildtype, GC
- Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.
- 0 A F1 G 17888 Z1 [ **Z**3 Z4 C 0 A 6 F1 Z5 □ G [ 26 C C 7 F3 T 800 Z8 □ Wildtype, CC Wildtype, GC
- Capture fluorescent products on addressable array and quantify each allele.



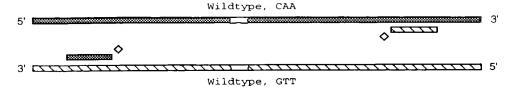
Heterozygous: Gly and Glu alieles.



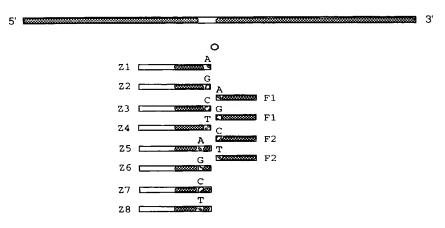
Heterozygous: Arg and Trp alleles.

#### PCR/ LDR: All alleles of a single codon

 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.

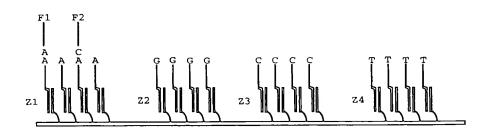


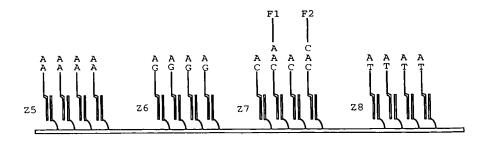
 Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3' Wildtype, GTT

 Capture fluorescent products on addressable array and quantify each allele.





Heterozygous: Gln and His alleles.

$$X = -OH$$
  $X^*, Y^* = -O(C=O)Z$   $-CO_2H$   $-CO_2$ 

W = protecting group, e.g. Boc, Fmoc

Z = activating group, e.g. imidazole (Im), p-nitrophenol (OPnp),hydroxysuccinimide (OSu), pentafluorophenol (OPfp)

PEG = oligo or poly(ethylene glycol), backbone  $(CH_2CH_2O)_n$   $\underline{n} = 6$  to 200 (can also be grown by anionic polymerization with )

WSC = water soluble carbodiimide

#### Functional group transformations/activation (as needed), $X \rightarrow X^*$ , $Y \rightarrow Y^*$

$$-OH \longrightarrow -O(CH_2)_nCO_2H$$
  $n = 1, 2$   
 $-OH \longrightarrow -O(C=O)NHCH_2CO_2H$ 

$$-OH \longrightarrow -O(C=O)CH_2NH_2$$

 $-OH \longrightarrow -O(C=O)Im$ 

 $-OH \longrightarrow -O(C=S)SCH_2(C=O)NH_2$ 

$$-CO_2H \longrightarrow -(C=O)NH(CH_2)_nNH_2$$
  $n = 2,6$ 

 $\begin{array}{cccc} -\text{CO}_2\text{H} & \longrightarrow & -(\text{C=O})\text{NH(CH}_2)_n\text{NH}_2 & \text{n} = 2,6 \\ -\text{CO}_2\text{H} & \longrightarrow & -(\text{C=O})\text{Z} \\ -\text{NH}_2 & \longrightarrow & -\text{NH(C=O)(CH}_2)_n\text{CO}_2\text{H} & \text{n} = 2,3 \end{array}$ 

#### Covalent linkage, X\* + Y\*

$$\begin{array}{lll} -\mathrm{CO_2H} + & \mathrm{H_2N-} + \mathrm{WSC} + \mathrm{HOSu} & \longrightarrow & -(\mathrm{C=O})\mathrm{NH-} \\ -\mathrm{OH} & + & \mathrm{Im}(\mathrm{C=O})\mathrm{Im} + \mathrm{H_2N-} & \longrightarrow & -\mathrm{O}(\mathrm{C=O})\mathrm{NH-} \end{array}$$

$$\begin{array}{c|c}
Cl & -O \\
-OH + N & -Cl & -N \\
N & -Cl & + H_2N - -N \\
Cl & -OH + H_2N - -N & -N \\
N & -N & -N \\
Cl & -OH - N & -N \\
N & -N & -N \\
N$$

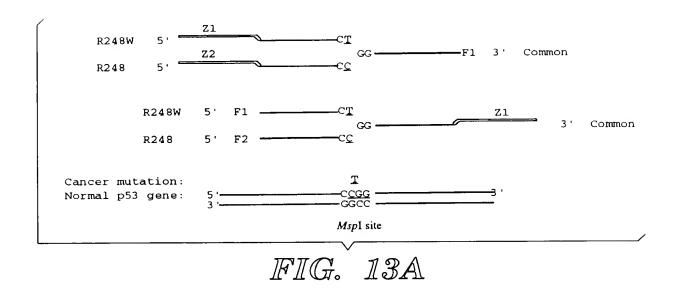
$$-OH + O=C=N- \longrightarrow -O(C=O)NH-$$

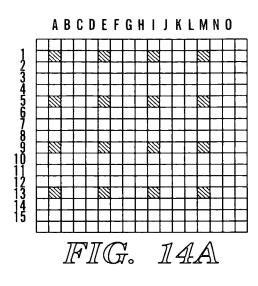
$$-O(C=S)SCH_2(C=O)NH_2 + H_2N- \longrightarrow -O(C=S)NH-$$

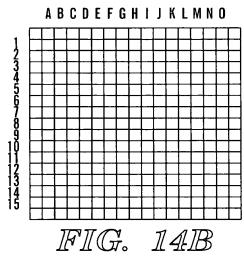
$$-OH + ClCH_2 \longrightarrow + HO- \longrightarrow -OCH_2CH(OH)CH_2O-$$

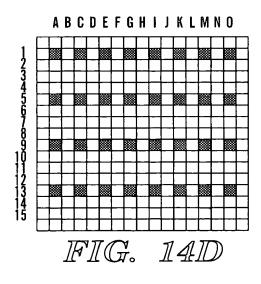
$$(NH-C)CH_2(C+C)CH(O+C)CH_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2(C+C)C_2$$

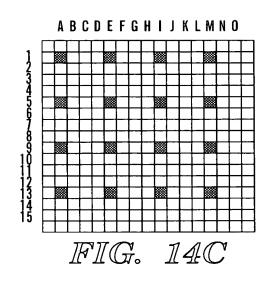
$$-OH \rightarrow -OCH_2(C=O)H + H_2N - + NaCNBH_3 \rightarrow -OCH_2CH_2NH -$$











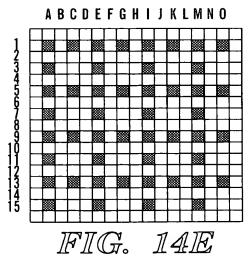


FIG. 15A

1st addition of unique 24mers.

FIG. 15B

2nd addition of unique 24mers.

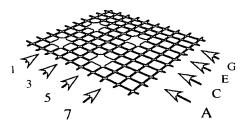
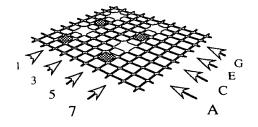
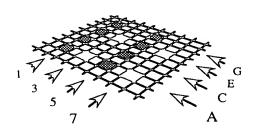
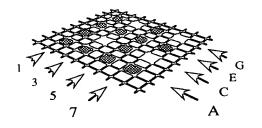


FIG. 15C 3rd addition of unique 24mers.



 $\mathbb{F}\mathbb{I}\mathbb{G}$ ,  $\mathbb{I}5\mathbb{D}$  4th addition of unique 24mers.





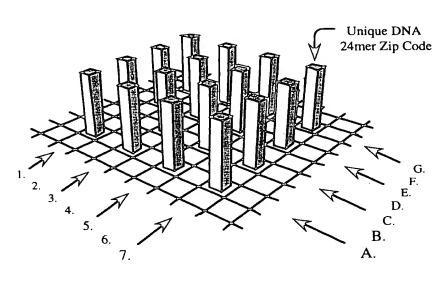


FIG. 15E

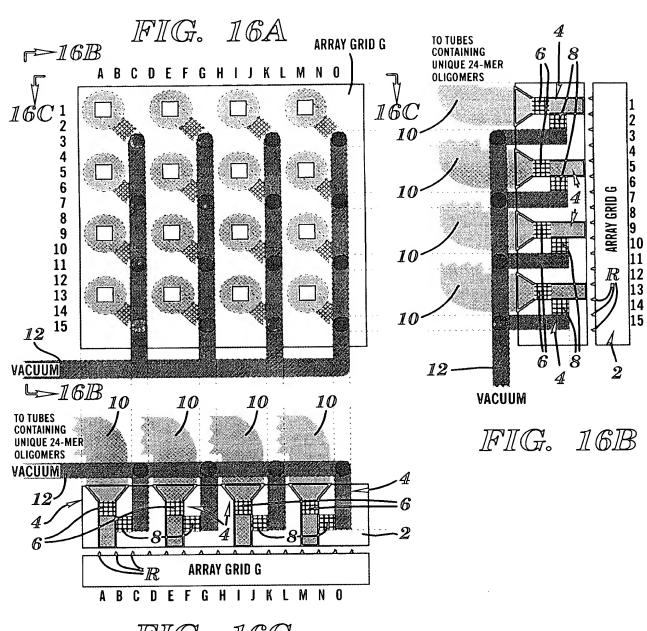


FIG. 16C



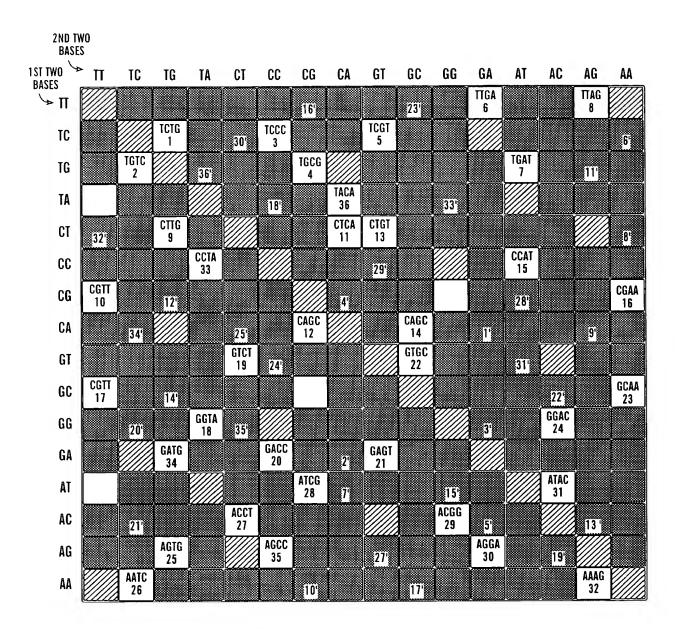


FIG. 17



1st Tetramer addition (columns)

| 4 |   | <br>_ | _ | <br> | <br> |  |
|---|---|-------|---|------|------|--|
|   | 1 | 2     | 3 | 4    | 5    |  |
|   | 1 | 2     | 3 | 4    | 5    |  |
|   | 1 | 2     | 3 | 4    | 5    |  |
|   | 1 | 2     | 3 | 4    | 5    |  |
|   | 1 | 2     | 3 | 4    | 5    |  |
|   |   |       |   |      |      |  |

FIG. 18A

4th Tetramer addition (rows)

| 2 | 2 | 2 | 2 | 2 |
|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 |
| 6 | 6 | 6 | 6 | 6 |
| 5 | 5 | 5 | 5 | 5 |
| 4 | 4 | 4 | 4 | 4 |

FIG. 18D

18/34

2nd Tetramer addition (rows)

| 6 | 6 | 6 | 6 | 6 |   |
|---|---|---|---|---|---|
| 5 | 5 | 5 | 5 | 5 |   |
| 4 | 4 | 4 | 4 | 4 |   |
| 3 | 3 | 3 | 3 | 3 |   |
| 2 | 2 | 2 | 2 | 2 | - |

FIG. 18B

5th Tetramer addition (columns)

| 6 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 6 | 1 | 2 | 3 | 4 |
| 6 | 1 | 2 | 3 | 4 |
| 6 | 1 | 2 | 3 | 4 |
| 6 | 1 | 2 | 3 | 4 |

FIG. 18E

3rd Tetramer addition (columns)

| 3 | 4 | 5 | 6 | 1 |
|---|---|---|---|---|
| 3 | 4 | 5 | 6 | 1 |
| 3 | 4 | 5 | 6 | 1 |
| 3 | 4 | 5 | 6 | 1 |
| 3 | 4 | 5 | 6 | 1 |

FIG. 18C

6th Tetramer addition (rows)

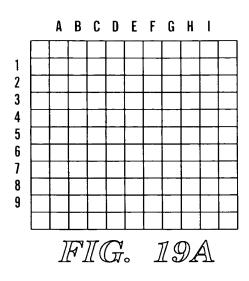
| 3 | 3 | 3 | 3 | 3 |
|---|---|---|---|---|
| 2 | 2 | 2 | 2 | 2 |
| 1 | 1 | 1 | 1 | 1 |
| 6 | 6 | 6 | 6 | 6 |
| 5 | 5 | 5 | 5 | 5 |

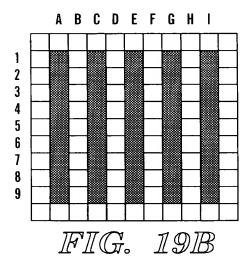
FIG. 18F

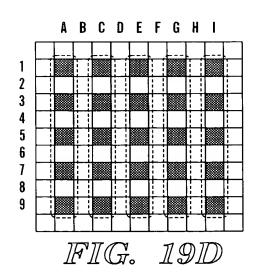
Addressable array with full length PNA 24mers

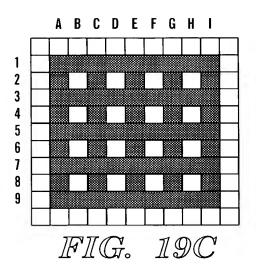
|      |          | ſ |             |             |             | -           |  |
|------|----------|---|-------------|-------------|-------------|-------------|--|
| 1-6- | 3-2-6-3  |   | 2-6-4-2-1-3 | 3-6-5-2-2-3 | 4-6-6-2-3-3 | 5-6-1-2-4-3 |  |
|      |          |   |             |             |             |             |  |
| 1-5- | 3-1-6-2  |   | 2-5-4-1-1-2 | 3-5-5-1-2-2 | 4-5-6-1-3-2 | 5-5-1-1-4-2 |  |
|      | Î        |   |             |             |             |             |  |
| 1-4- | -3-6-6-1 |   | 2-4-4-6-1-1 | 3-4-5-6-2-1 | 4-4-6-6-3-1 | 5-4-1-6-4-1 |  |
|      |          |   |             |             |             |             |  |
| 1-3- | -3-5-6-6 |   | 2-3-4-5-1-6 | 3-3-5-5-2-6 | 4-3-6-5-3-6 | 5-3-1-5-4-6 |  |
|      |          |   |             |             |             |             |  |
| 1-2  | -3-4-6-5 |   | 2-2-4-4-1-5 | 3-2-5-4-2-5 | 4-2-6-4-3-5 | 5-2-1-4-4-5 |  |
|      |          |   |             |             |             |             |  |

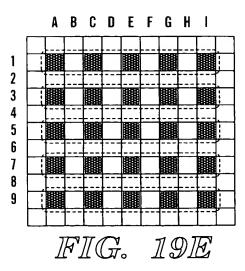
FIG. 18G

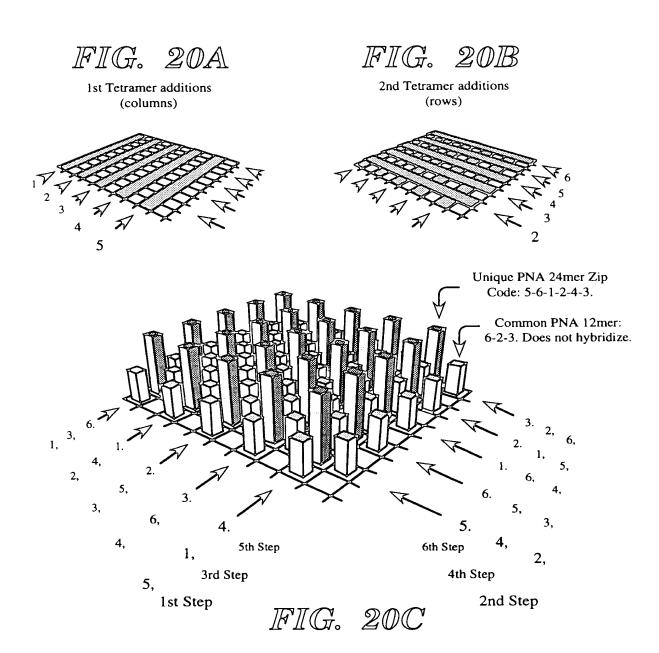


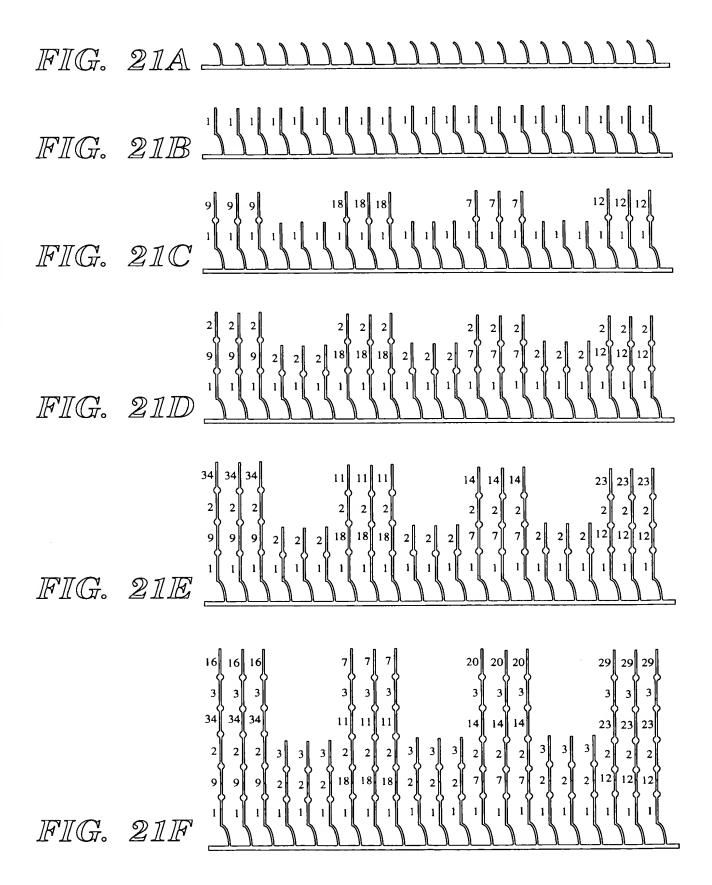


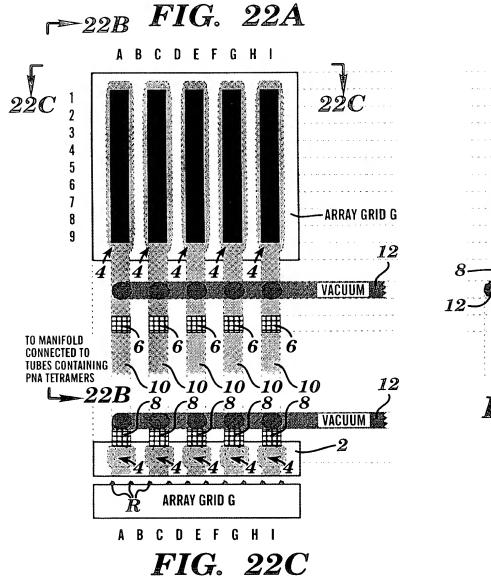












2 4

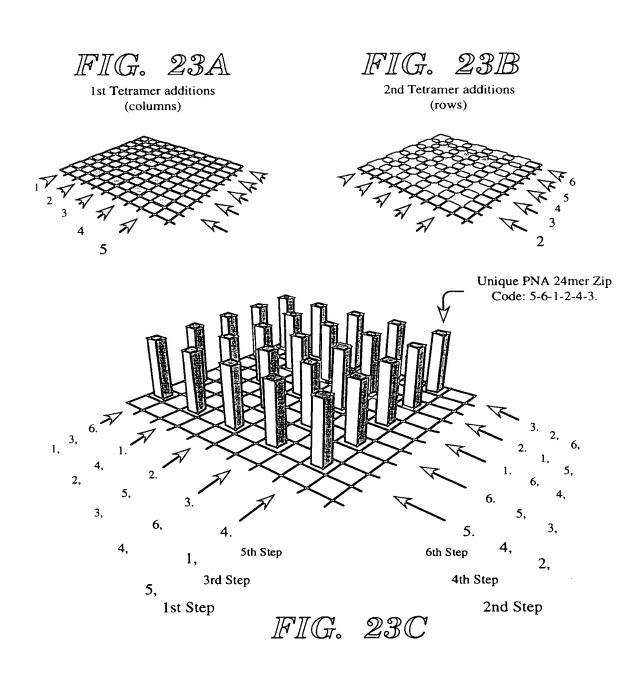
5 0 OIRS AVABLE

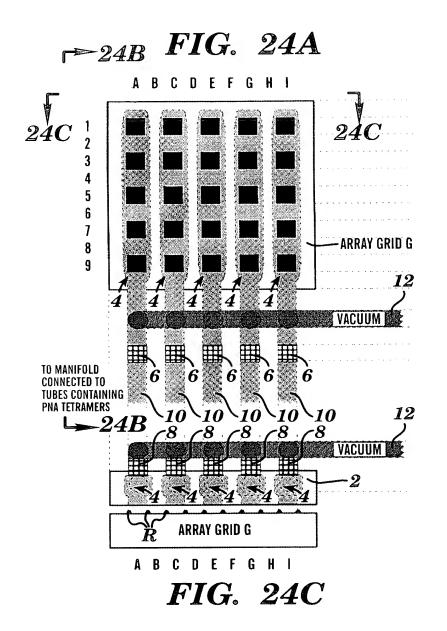
1 2

3 4
5 6
7 8
9

10 MANIFOLD
CONNECTED TO
TUBES CONTAINING
PNA TETRAMERS

FIG. 22B





2 4

1 2

3 4

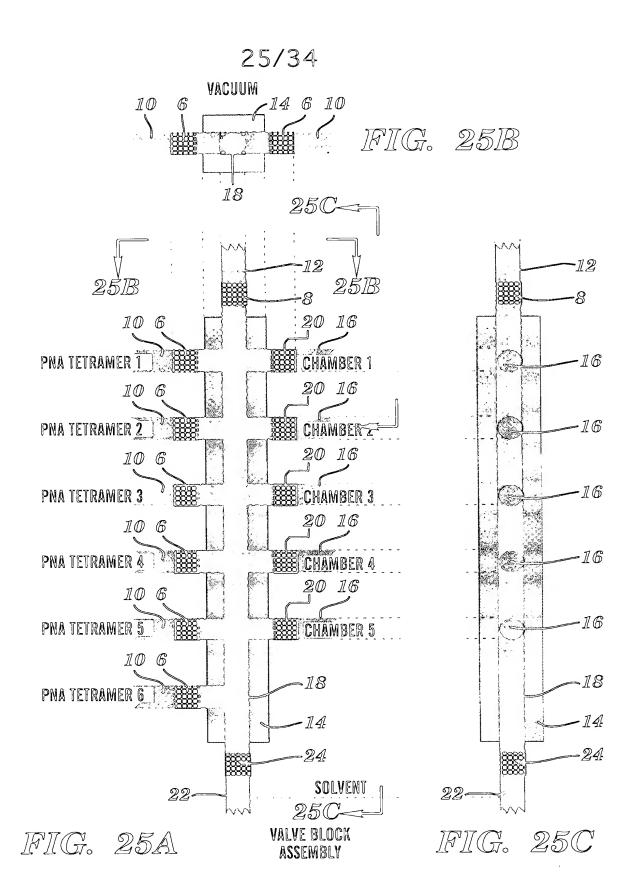
5 6

7 8

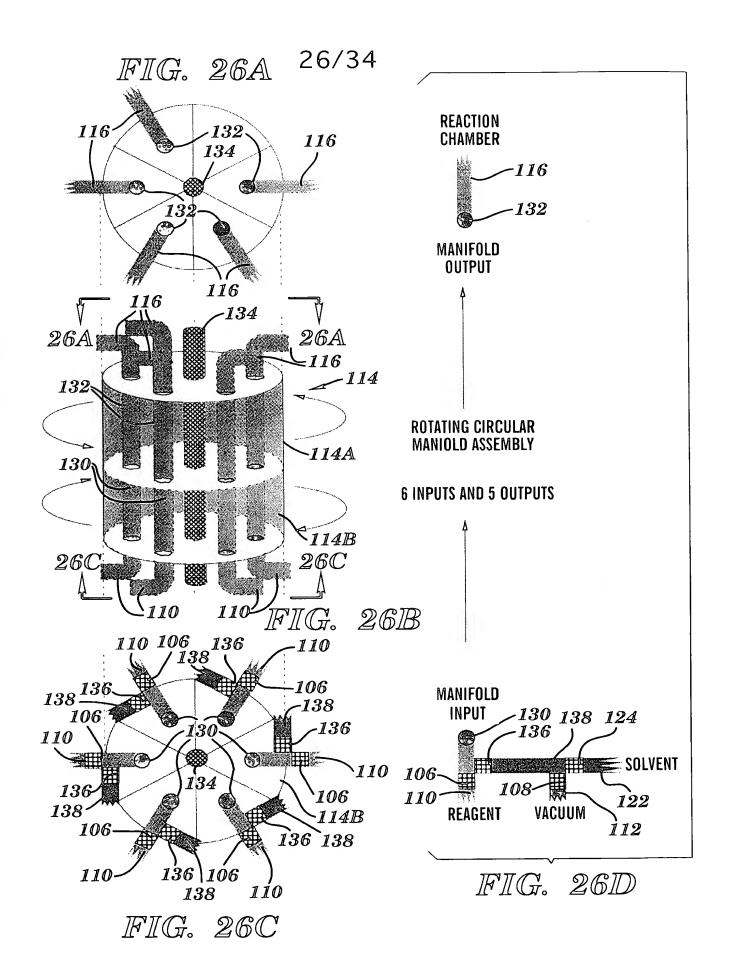
9 9

10 TO MANIFOLD CONNECTED TO TUBES CONTAINING PNA TETRAMERS

FIG. 24B



6 INPUTS AND 5 OUTPUTS



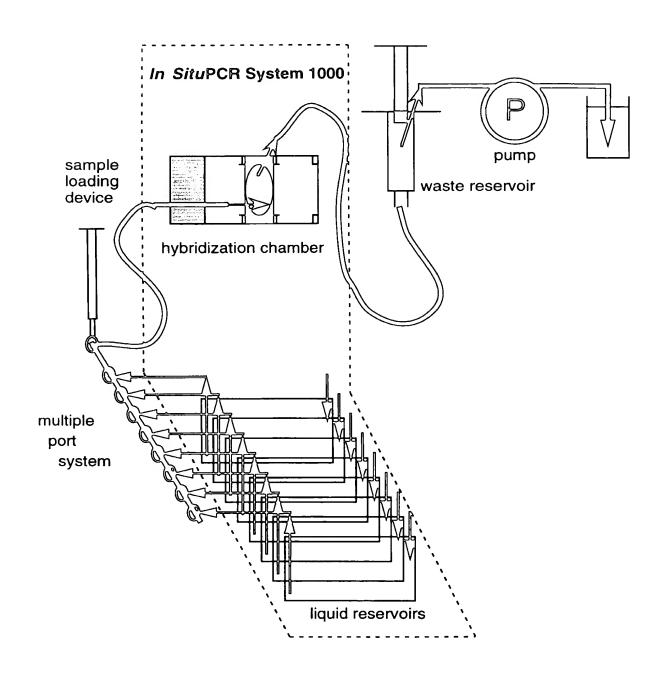


FIG. 27

-C00H; PR0BE 12

-COOH; PROBE 14

-NH2; PROBE 12

-NH2; PROBE 14

2% EGDMA

2% HDDMA

4% EGDMA

1

$$\begin{array}{c} H_{2}C \\ C - CH_{3} \\ O = C \\ O \\ CH_{2} \\ CH_{2} \\ CH_{2} \\ CH_{2} \\ CH_{3} \\ N \sim 5 \end{array}$$

FIG. 32

$$\begin{array}{c} H_2C \\ \vdots \\ C-CH_3 \\ O=C \\ \vdots \\ CH_2 \\ CH_2 \\ \vdots \\ CH_2 \\ CH_2 \\ \vdots \\ CH_2 \\ CH_2 \\ \vdots \\ CH_2 \\ \vdots$$

FIG. 33

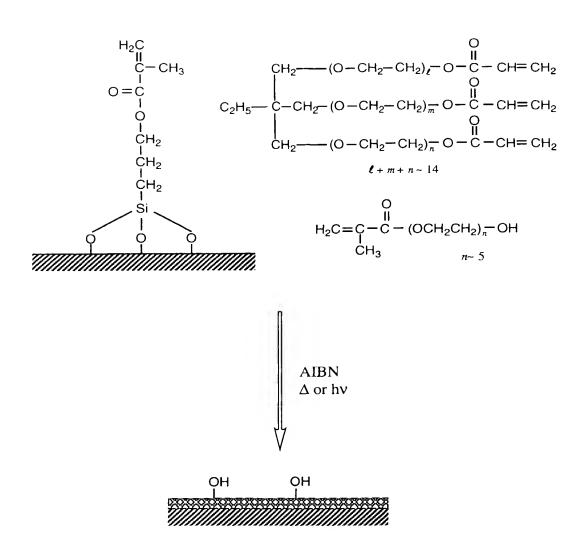


FIG. 34